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MÉMOIRES ORIGINAUX

Ultrastructure of *Trypanosoma lewisi*, Localization and Alterations in Rat Liver

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Summary.

Fine structure of *Trypanosoma lewisi* in rat liver has been studied by electron microscopy 14 days after infection. Various sections examined showed the parasites to be located in liver's sinusoids or space of Disse.

Electron micrographs demonstrate that the contact between the flagellum and the pellicle at the attachment zone is maintained through a desmosome-like substance, while a peculiar section illustrates a fusion of two cells which tangentially depict lattice-like surface and cross-striation of flagellar bundle.

Parasites found in sinusoids showed double-membranes, double coiled, DNA-rich kinetoplast with cristae present in basal portion and a prokinetosome parallel in position to the kinetosomal plate which is completely separated from the kinetosome that fastens the flagellum to it like a zipping hook. The positions of these flagellar organelles to the kinetoplast indicate that the kinetosome replication is initiated beyond the kinetoplast division. It is not certain whether the four vacuole-like tubules located in the cytoplasm parallel to kinetosome complex are functionally related to impulse coordination. However, they appear to be involved in pinocytotic activity.

Striking features of the parasite in the sinusoid and space of Disse include dense cytoplasmic granules, free ribosomes, nucleus with double membranes and nuclear pores, variable size vacuoles, dense bodies, and rough surface endoplasmic reticulum stretching from the

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anterior region and bypassing the golgi area all the way to the posterior end of the kinetoplast. The existence of a continuous highly organised relationship between the external nuclear membrane, endoplasmic reticulum and other cytoplasmic contents is demonstrated in the micrographs of the 'liver parasites'. The complexity observed and reported here is further documented by (*a*) that external mitochondrial membrane is shown to be in contact with sub-pellicular microtubules, (*b*) unity and continuity of the mitochondria with an agranular secretory reticulum exist, and (*c*) that the elongated mitochondrial extension from the kinetoplast deep into the cytoplasm confirms a previous report that it originates from the kinetoplast. The presence of abundant free ribosomes in the parasite convincingly indicates that the organism is progressively synthesizing more protein when trapped in the tissue.

The investigation also demonstrates that when *Trypanosoma lewisi* where found enclosed in hepatic sinusoid and space of Disse, pathological alterations in the parasite occurred. Inflammation of flagellum at the base and the entire cytoplasmic content of the mid-posterior portion of the organism also occurred.

Liver cells parasitized by the trypanosomes showed dilated cisternae of endoplasmic reticulum, swollen mitochondria, fatty infiltrations, dense bodies, vacuoles of different sizes and numerous clusters of ferritin granules. The parasite increased rhythmic activity of locommotion might have enhanced these alterations. Hydrolytic effect of numerous lysosomes of the liver cells might have accelerated the alterations. On the other hand the accumulation of excessive metabolic products of the parasites themselves could also have exerted alterable effect on the liver cells and the sinusoids. Lysosomes or microbodies found in the various liver trypanosomes were possibly spontaneously released by rupture due to injured or dying cells.

Résumé.

Ultrastructure de* T. lewisi, *localisation et altérations hépatiques chez le rat.

La microstructure de *Trypanosoma lewisi* dans un foie de rat a été étudiée au microscope électronique quinze jours après l'infection. Les études faites sur différentes sections ont montré que les parasites se logent dans le foie, dans la sinusoïde ou l'espace lymphatique.

Comme l'ont montré les micrographies électroniques, le contact qui existe entre le flagelle et la membrane, à leur zone de fixation, est maintenu par une substance d'apparence desmosomique, tandis qu'une section en particulier donne l'exemple d'une fusion de deux cellules qui, de manière tangentielle, forment une surface grillagée et montrent aussi un faisceau flagellaire strié transversalement.

Les parasites trouvés dans la sinusoïde ont fait apparaître un cinétoplaste riche en ADN, à membranes et filaments doubles, présentant des crêtes dans la portion basale ainsi qu'un procinétosome parallèle à la plaque cinétosomale ; celui-ci est complètement séparé du cinétosome qui forme un lien avec le flagellum comme le crochet d'une fermeture-éclair. La position de ces organelles flagellaires par rapport au cinétoplaste indique que la multiplication cinétosomique s'amorce au-delà de la division cinétoplastique. Il n'est pas certain que les quatre tubules d'apparence vacuolaire placées dans le cytoplasme parallèlement au complexe cinétosomal aient une relation fonctionnelle avec la coordination de la poussée motrice. Toutefois, ils semblent liés à l'activité pinocytotique.

On relève chez le parasite logé dans la sinusoïde et l'espace lymphatique les caractéristiques suivantes : des granules cytoplasmiques denses, des ribosomes libres, un noyau à membranes doubles et pores nucléaires, des vacuoles de taille variable, des corps denses et un réti-

culum endoplasmique à surface rugueuse s'étendant de la région antérieure à l'extrémité postérieure du cinétoplaste, en contournant la zone de Golgi. Les micrographies des parasites du foie ont prouvé l'existence de rapports continus hautement organisés entre la membrane nucléaire externe, le réticulum endoplasmique et une autre substance cytoplasmique. La complexité des phénomènes observés est soulignée par les faits suivants : (*a*) la membrane mitochondriale externe est en contact avec les microtubules subpelliculaires ; (*b*) il y a unité et continuité de la mitochondrie avec un réticulum sécréteur agranulaire ; (*c*) la surélongation mitochondriale, qui prend son origine dans le cinétoplasme, s'encastre profondément dans le cytoplasme ; ceci confirme les données d'un rapport antérieur selon lequel l'élongation prend son origine dans le cinétoplaste. La profusion de ribosomes libres dans le parasite indique que l'organisme synthétise progressivement davantage de protéines lorsqu'il est bloqué dans le tissu.

Ces travaux de recherche ont également démontré que lorsque *Trypanosoma lewisi* se trouve enfermé dans la sinusoïde hépatique et dans l'espace lymphatique le parasite subit une détérioration pathologique. Il y eut également inflammation du flagelle à la base et frappant toute la substance cytoplasmique de la portion centrale et postérieure de l'organisme.

Les cellules du foie infectées par les trypanosomes accusaient des citernes dilatées du réticulum endoplasmique, une mitochondrie enflée, des infiltrations adipeuses, des corps denses, des vacuoles de taille variable et de nombreux amas de granules de ferritine. Le rythme accru de l'activité locomotrice provoqué par le parasite aura peut-être aggravé ces détériorations. L'effet hydrolitique des nombreux lysosomes des cellules du foie aura peut-être accéléré ces modifications. D'autre part, l'accumulation excessive de produits métaboliques des parasites eux-mêmes aura peut-être causé des modifications des cellules sinusoïdales du foie. Les lysosomes ou microcorps trouvés dans les différents trypanosomes du foie ont été peut-être libérés spontanément à la suite d'une rupture de cellules lésées ou mourantes.

Introduction

The advent of better techniques for electron microscopy has in recent years contributed to our knowledge of trypanosomes and other parasites to some degree (Meyer et al., 1958 ; Vickerman, 1962, 1969 ; Trager, 1963 ; Anderson and Ellis, 1965 ; and Wright et al., 1969, 1970). Today, only a few of the institutions located in the endemic African countries suffering from trypanosomiasis can yet afford the costs of highly sophisticated research and expensive equipments.

In the family trypanosomatidae such as *Leishmania donovani, Trypanosoma lewisi, Trypanosoma brucei, Trypanosoma cruzi,* and *Trypanosoma rhodesiensi,* some morphological similarities of cilia and flagellar substructure have been reported, while to an extent basic pattern variations in morphological features such as in flagellar microtubules and kinetoplasts have been demonstrated (Meyer and Porter, 1954 ; Clark and Wallace, 1960 ; Rudzinska et al., 1964 ; and Anderson and Ellis, 1965).

Ultrastructure of *Trypanosoma cruzi* in myocardium of white mice was recently studied (Sanabria and Aristimuno, 1970). Morphological changes and microscopic lesions of liver in acute chagas disease of man have been reported (Andrare and Lopes, 1963). In view of these and other reports, many of the unsolved physiological and pathological questions on the host-parasite relationship still confuse research investi-

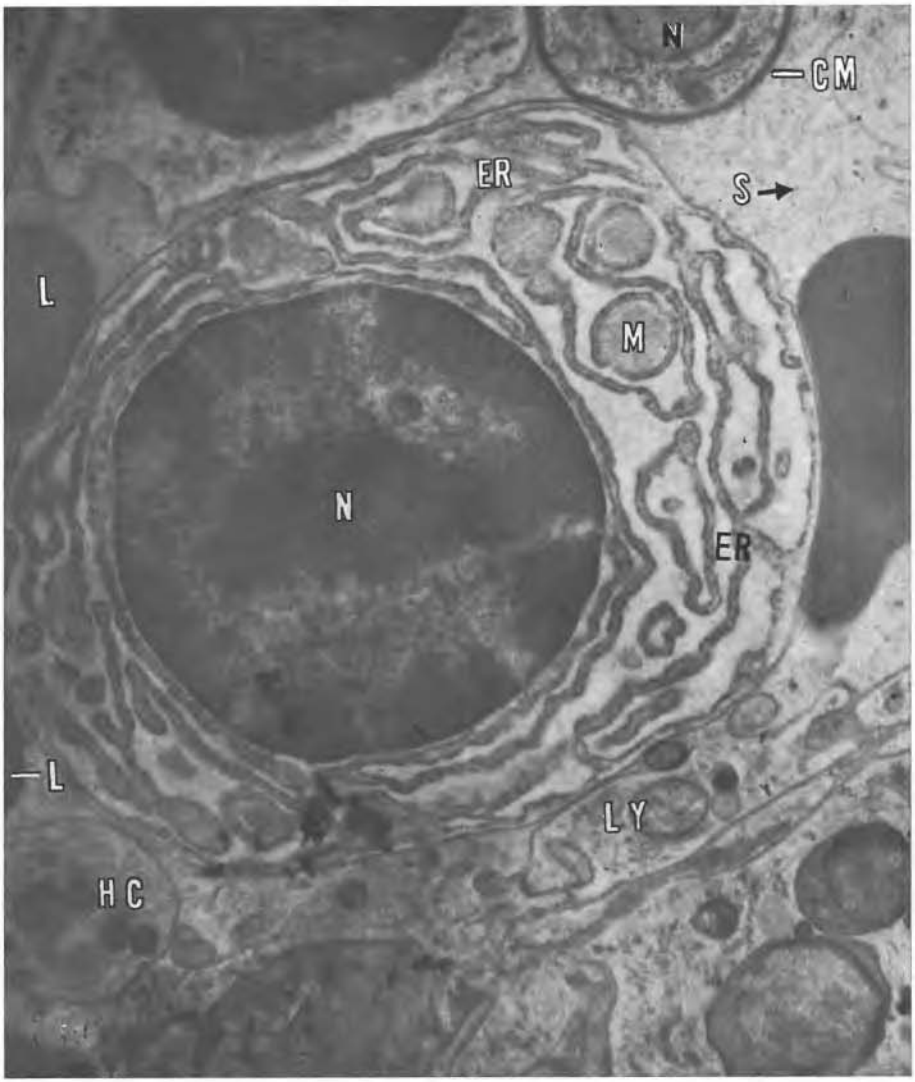

FIG. 1 *a*. — A magnification of liver section with nucleus centrally located, and surrounded by mitochondria, rough endoplasmic reticulum. The top is bordered by sinusoid where partial nuclear portion of the parasite is located. Vissible are red blood cell and hepatic cells. × 12,320

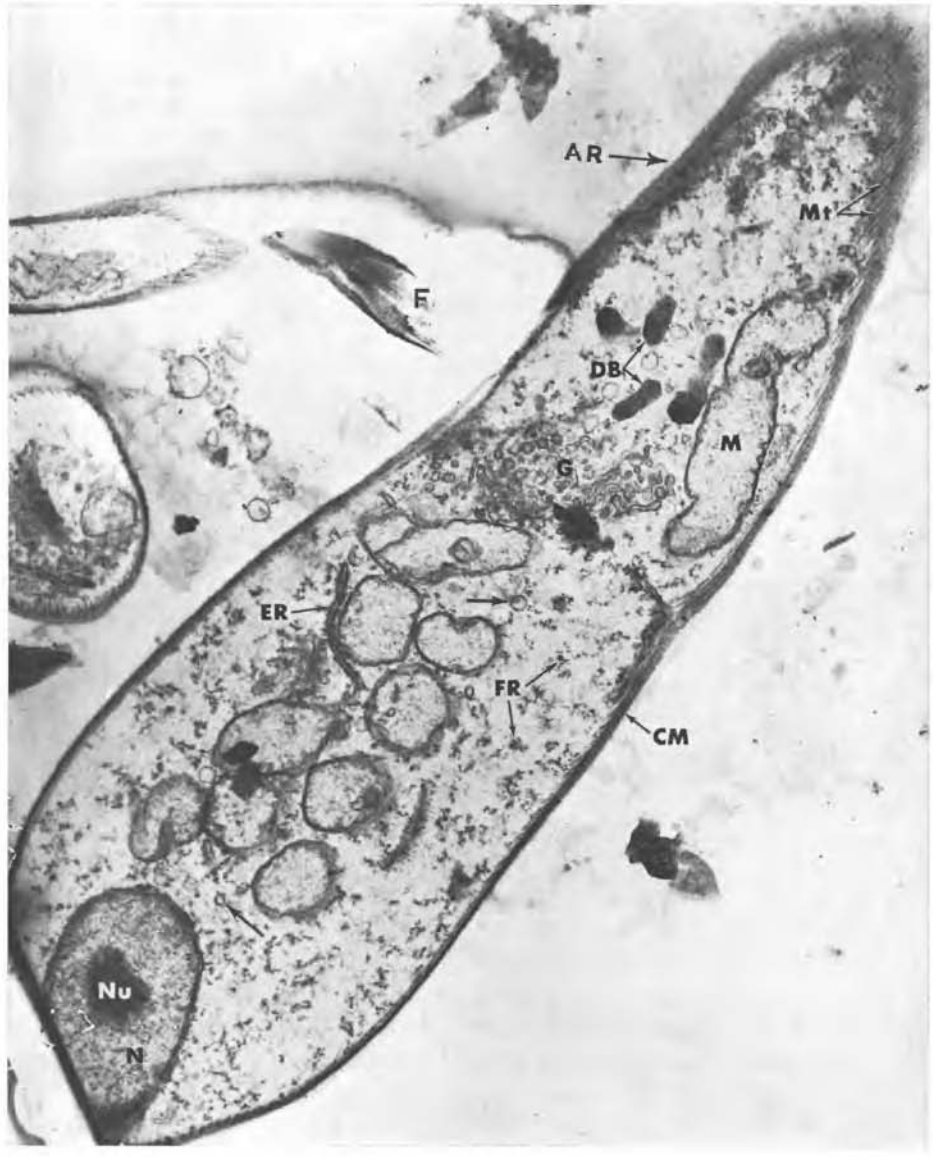

FIG. 1 *b*. — Longitudinal section of *Trypanosoma lewisi* inside a hepatic dinusoid showing fine structure of area between nucleus and golgi. Dense bodies, mitochondrial extension, rough endoplasmic reticulum, pellicular microtubules, free ribosomes and broken flagellar. × 16,800

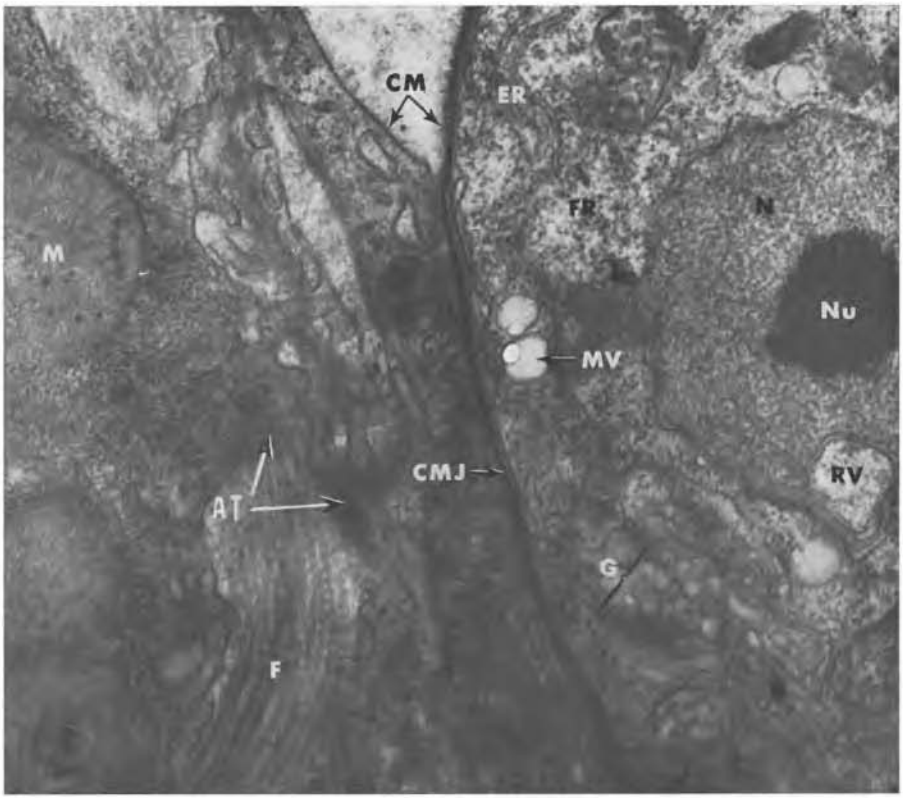

FIG. 2. — Two Trypanosomes inside the hepatic sinusoid, united by their outer membranes forming a cytoplasmic bridge (CMJ) arrow. Nuclear and golgi zones of both cells areas show agranular secretory reticulum, membrane bound vessicle, rough endoplasmic reticulum and cytoplasm studded with dark free ribosomes. The left parasite shows a single cell membrane (arrow), mitochondria, and a lattice-like tangential section of flagellum and its basal attachment zone (AT) arrow. × 36,000

gators. As far as it is known, the report here for the first time throws light on the fine structure and modification of *Trypanosoma lewisi* in rat liver tissue and some morphological and pathological observations found in the infected liver tissue by the parasite.

Materials and Methods

Uninfected Sprague-Dawley strain rats were injected intraperitoneally with saline blood suspension of *Trypanosoma lewisi* obtained from a rat which had been previously infected. An estimated microscopic quantity of 2,000 trypanosomes was used for each infection.

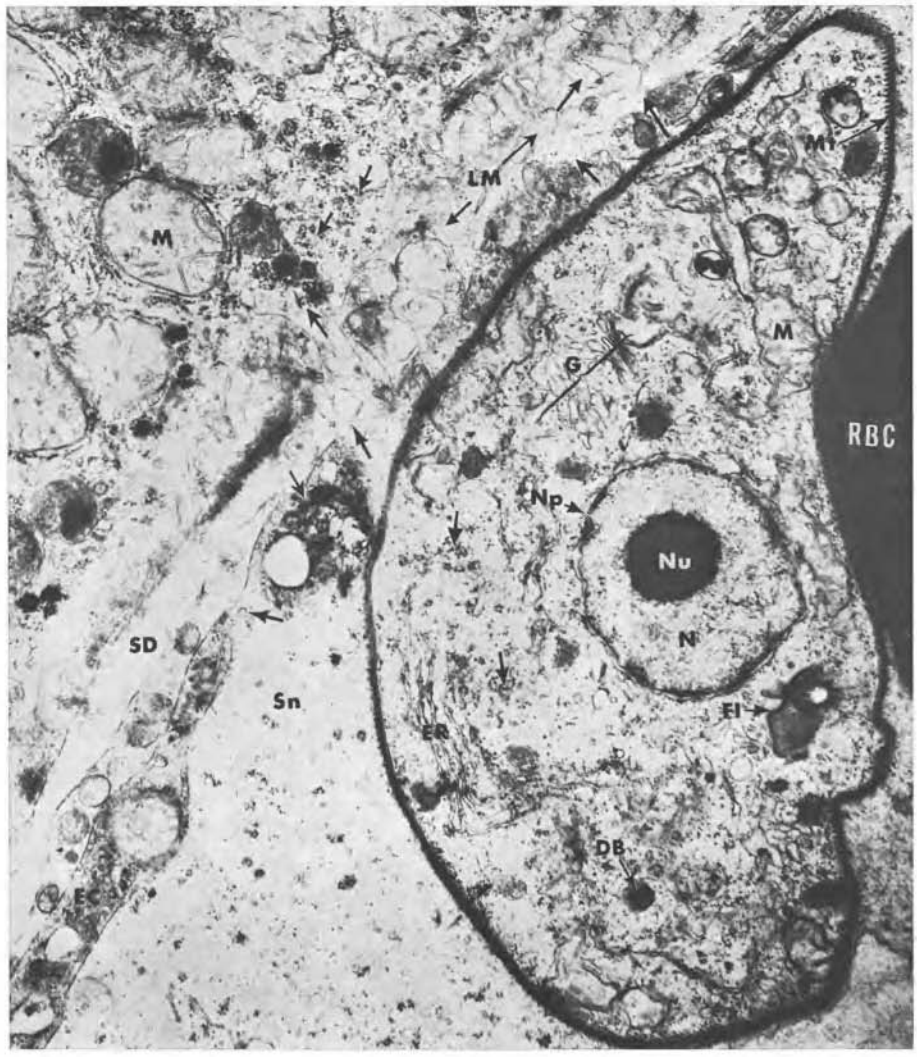

FIG. 3. — A transverse section through mid-posterior region of the parasite in lever sinusoid. The trypanosome exerts pressure on the endothelium (EC) of the Kupffer cells by blocking some liver cell fenestrations (arrow) and compressing many short microvilli (LM). The resultant effect is an occlusion of adjacent space of Disse on one side. Sub pellicular microtubules, golgi zone, prominent nucleus, nuclear pore, dense bodies, endoplasmic reticulum, mitochondria, ribosomes and lipid droplets are well displayed in the cell and the liver tissus. × 16,800

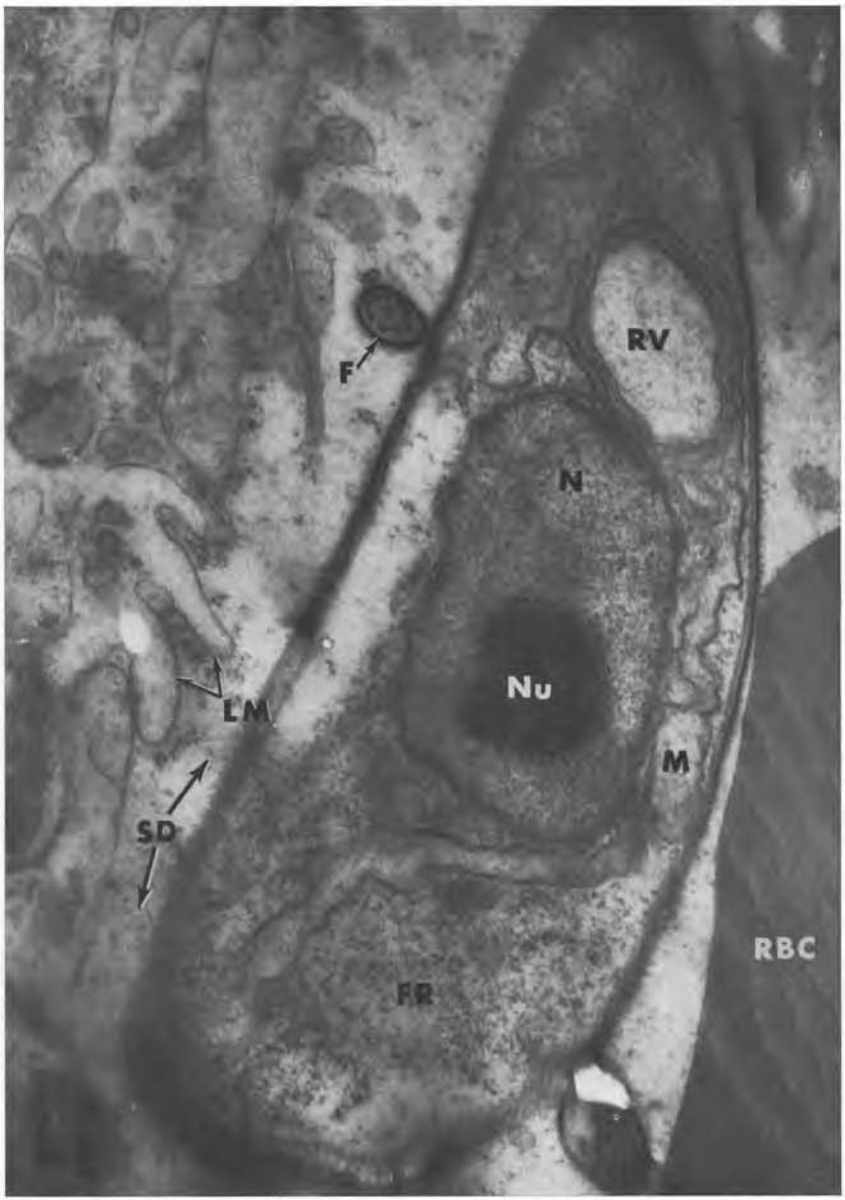

FIG. 4. — Transverse section of the anterior body nuclear and flagellum area of the parasite trapped within space of Disse in the liver. Prominently displayed is a continuous mitochondrial extension leading to and forming the third external layer of a smooth membraneous secretory reticulum (RV) behind the nucleus. Dense cytoplasmic free ribosomes and flagellar tubules are shown. × 30,400

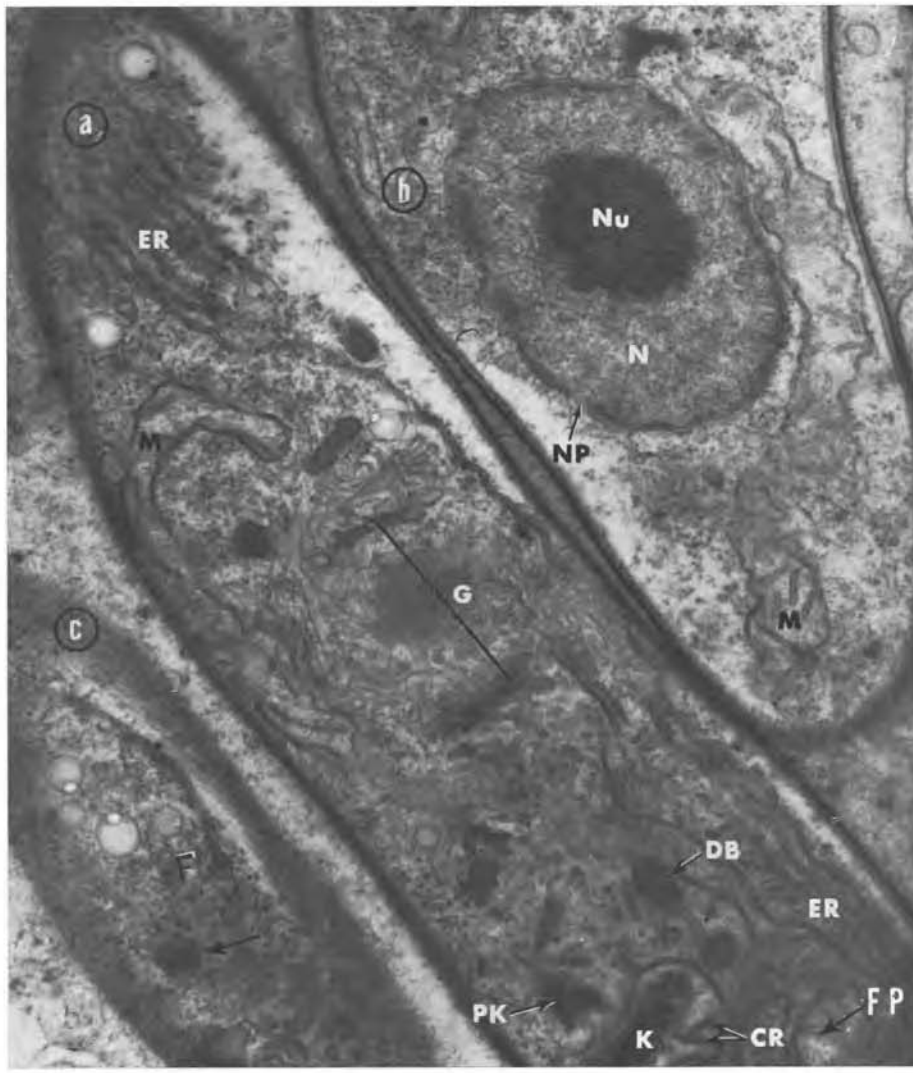

FIG. 5. — Trypanosomes in liver space of Disse. (*a*) Middle figure in an oblique longitudinal section of posterior region of the cell demonstrating an elongated mitochondria, gorgeous endoplasmic reticulum continuous to the north and south of the polarized golgi. Posteriorly are two dense bodies linked by a narrow canal and a shield-like prokinetosome (PK) located above the cristae contained kintoplast. (*b*) Top figure is the middle nuclear curvature section of the parasite showing elongated mitochondria, dense nucleolus, nucleus with nuclear pore. (*c*) bottom left represents a flagellar system. × 35,200

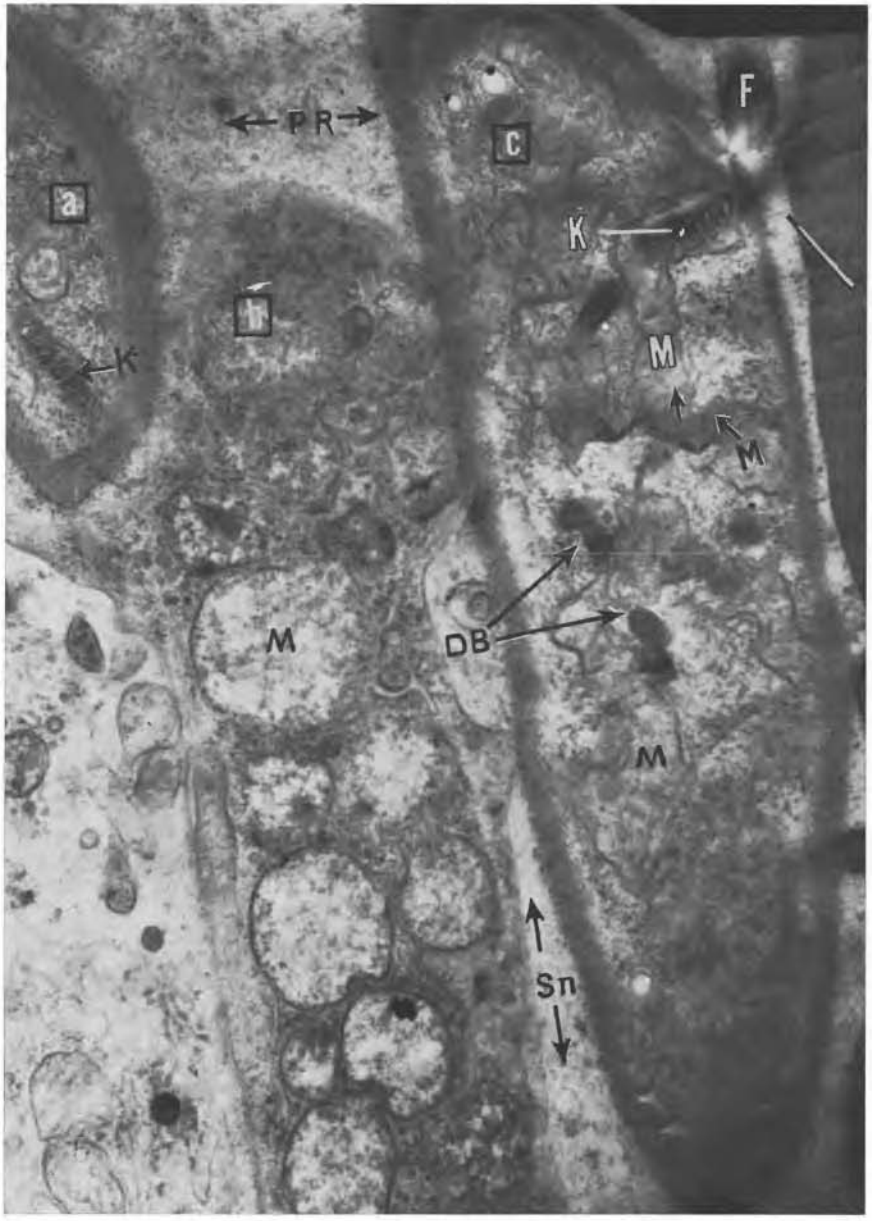

FIG. 6

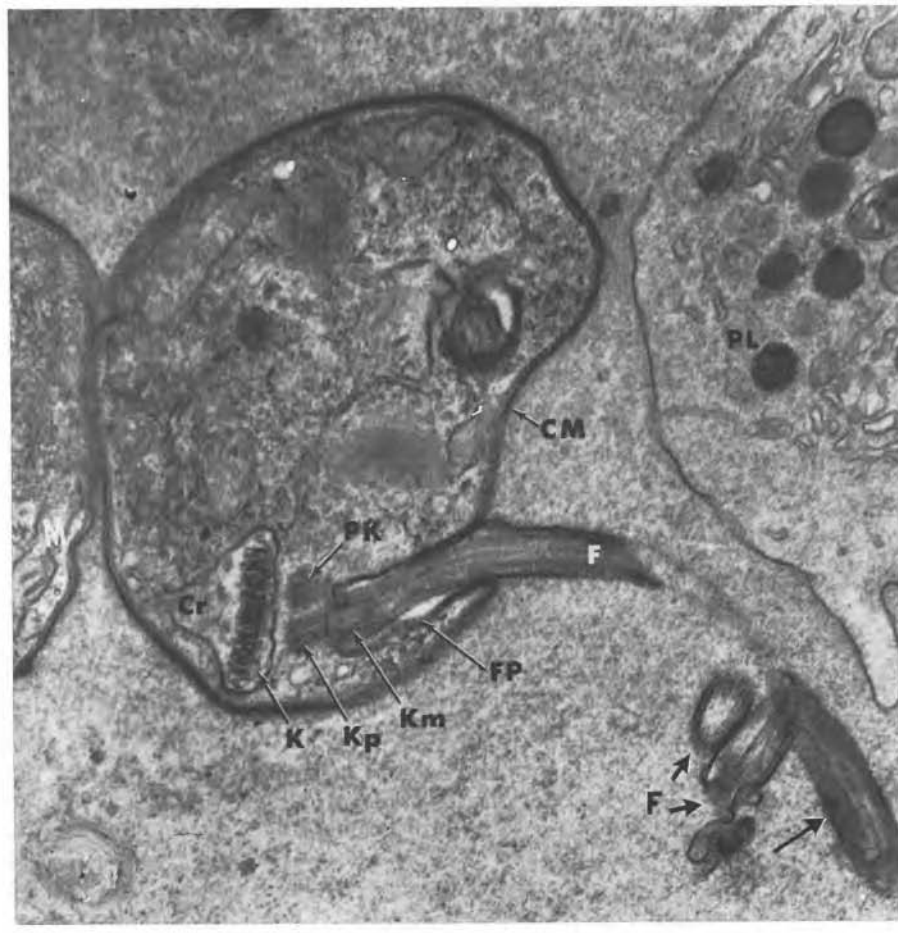

FIG. 7. — Cross section through the kinetoplast showing the zone of flagellan attachment. The two parasites are trappeld closely together across inside of hepatic sinusoid with its parallel paraxial rod and axoneme almost completely fills the flagellar pockett (FP). Prominent are platelets, mitochondria, endoplasmic reticulum and free ribosomes. The prokinetosome (PK) which is situated above the double coiled kinetoplast (K) is also parallel to kinetosomal plate (KP). The kinetosomal plate appears separated from the kinetosome (Km) to which the flagellar is hooked. Unit membrane continuity and membraneous invagination are well exemplified. × 34,600

FIG. 6. — Longitudinal sections of parasites possessing thich unit limitting membranes are phagocytized by Kupffer cell inside of sinusoid. The micrographs demonstrate abundant dense bodies and swollen mitochondria in (*b*) and (*c*) cells, and kinetoplasts in (*a*) and (*c*) cells. A continuous but winding mitochondrial extension stretching from the anterior end and running posteriorly uniting with the external membrane of kinetoplast in (*c*) organism is shown. Attachment zone (arrow) of the flagellar is vissible. The posterior end of the trypanosome (*b*) appear bulging and inflammed with extensive membraneous protrusions to adjacent parasites and hepatic cell. × 19,110

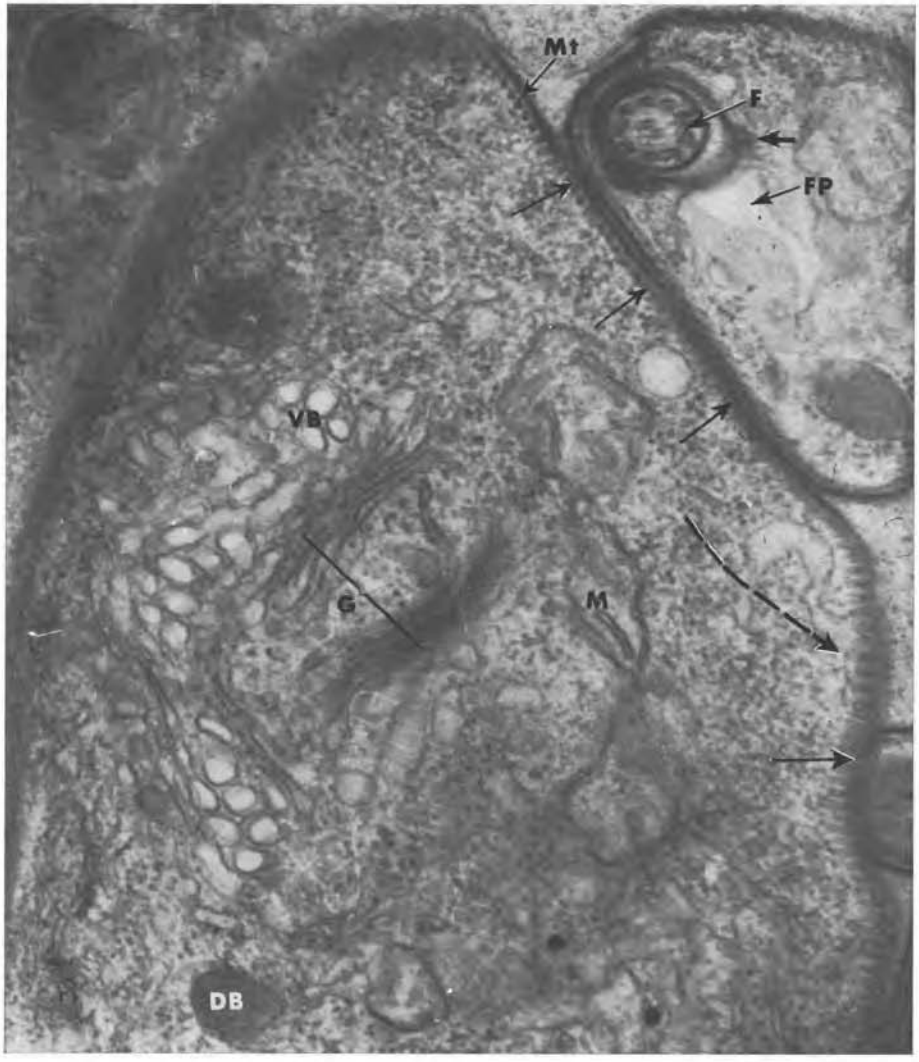

FIG. 8. — Longitudinal transverse section demonstrating polarized golgi apparatus with vessicular bodies and flagellar shaft attachment region to the cell. Distictively shown are the cells, sub pellicular microtubules, the flagellum 9 double peripheral and 2 central microtubules, free ribosomes, dense bodies and mitochondria. The flagellar pocket shows membraneous invagination. Parallel arrows indicate points of desmosome-like attachment zone between the continuous flagellar body extension and the cell. Also the three fused peripheral arrangement of the fibrillar microtubules represent the presence of nine peripheral fibrils in the basal body and centriole (arrow). × 39,400

FIG. 9. — Portion of longitudinal section of figure 8 showing nucleus and the anterior area. The parasite is enclosed in liver space of Disse with flagellar undulating portion broken off. Nuclear pore, free ribosomes, mitochondrial extension and membrane bound vessicle are demonstrated. × 44,100

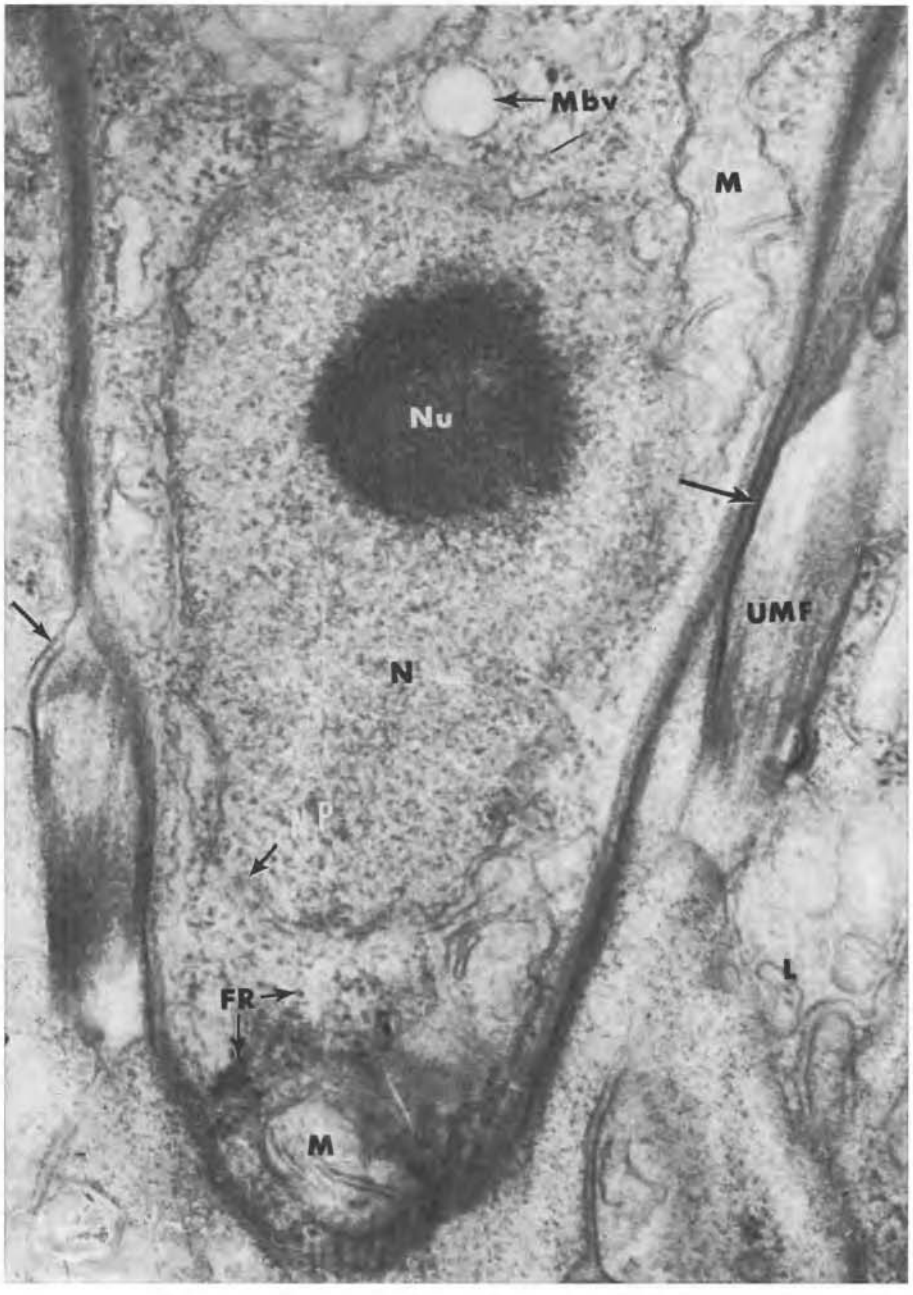

FIG. 9

After 14 days each rat was sacrificed by a rapid twist of the head and the liver sections of one cubic millimeter were instantly immersed in a 50 ml beaker containg 1% isotonic phosphate buffered osmium tetroxide fixative solution (Millonig, 1961) at pH 7.3 for 2 hours. The fixation was followed by repeated washing with the buffer solution for 1 hour each at 4 degree centigrade. The tissues were thereafter dehydrated through a graded series of absolute alcohol at room temperature. Clearance of the alcohol was accomplished by placing the sections in propylene oxide (two changes) for 15 minutes, after which they remained in a 1 : 1 mixture of propylene oxide and Epon for 4 hours, and in a 1 : 3 propylene oxide and Epon mixture for 12 hours in room temperature. The tissues were embedded in Epon mixture (Luft, 1961) dispenced in gelatin capsules. The blocks were cured in the oven at 60 degrees centigrade for 36 hours to effect excellent polymerization.

Ultrathin sections were cut with diamond knives on a Porter-Blue MT-2 Ultramicrotome. The sections were collected on 0.5% collodion-coated grids (Pease, 1964), and stained in aqueous uranyl acetate or lead citrate (Watson, 1958). The grids were then examined and photographed with an RCA EMU-2D electron microscope.

Results and Observations

Trypanosoma lewisi has been studied in rat 14 days after infection. Electron micrographs of the organism in liver tissue showed that it has a thick trilaminated cell membrane (figs. 1, 2, 6, 8, 9) which illustrates its continuity with the flagellar membrane forming an invagination at the region of attachment (fig. 7, 8). From the results, observation also indicates that the membrane of flagellar bundle has a desmosome-like contact with the cell surface at the attachment zone (fig. 4, 6 - 9 arrow). A similar flagellar adherence to the pellicle was reported by Anderson and Ellis (1965) in their studies of pure suspension of the organism. Among other sections is found a peculiar section, demonstrating a fussion of two parasites in the sinusoid through and unresolved layered limtting membrane surrounding each of the cells (fig. 2).

Further more, figures 7, 8, and 9 demonstrate that the distal and the flagellar pocket appear totally closed with the membraneous ends at the zone of attachment, while the desmosome-like linkage between the external membraneous layer of the parasite and the external membrane of the flagellum is virtually separated on one side (arrow fig. 9). The distal zone of attachment also forms an electron dense region (fig. 2, 9). Micrographs of the parasite phagocytized by the Kupffer cells show a tangential section of the flagellum with its lattice-like pattern and cross-striation appearing like a honeycomb with microtubules (fig. 2). A transverse section of the flagellum showing its unique 9 + 2 filaments is well displayed in the parasites trapped in the liver space of Disse (fig. 4, 8).

Inside hepatic sinusoid, a distinguished cross-section of the parasite posterior region through the kinetoplast shows presence of a prokinetosome parallel in position to kinetosomal plate, above the electron dense, double coiled kinetoplast containg DNA (fig. 7).

The micrograph also illustrates a continuity in the longitudinal zone of attachment where the kinetosome and its supporting kinetosomal plate zippingly fastens the flagellum to the kinetosome (fig. 7). The proximity of the kinetoplast to the prokinetosome and kinetosomal plate is significantly striking. An electron-dense kinetosomal plate appears completely separated from the kinetosome (fig. 7). While a dense matrix reminicent of an intra-flagellar structure is displayed in figure 8, the flagellar pocket appears to possess no sub pellicular microtubules. The only exception is the appearance of three microtubules which together form a cluster at the edge of the limitting membrane of the flagellar pocket (fig. 8 arrow). There are also four vacuole-like tubules located in the cytoplasm at the rigt evagination curvature of the flagellar pocket (fig. 7).

Two separate cross-sections of the parasite (fig. 7, 9) reveal short broken flagellar remnants which show clockwise symetry similar to the findings of Anderson and Ellis (1965). Again, it is demontrated that the kinetoplast is surrounded by double membrane and contains cristae in its basal portion (fig. 5, 7).

In this report, evidence of a uniform pattern of sub pellicular microtubules is presented in the flagellum tangential section (fig. 2). The tubules run into the cell cytoplasm. In addition, the longitudinal-transverse section through the posterior region of the cell testifies that a highly organized system of microtubules beneath the cell membrane exists (arrow fig. 3, 7, 8). The microtubules are found running singly and equidistant to one another. They are also parallel to the longitudinal axis of the cell and are found throughout the cell, but more predominantly seen in the posterior and middle region than the anterior region of the cell (fig. 3, 7, 8).

The parasite contains abundant vessicular bodies (fig. 1, 9). Between the kinetoplast and nucleus, numerous electron dense bodies have been observed particularly in the sections of the cells trapped in liver sinusoid and space of Disse (fig. 1, 3, 5). Different sections of the parasite in these spaces show that the cell,s nucleus is surrounded by a double membrane with nuclear pores. The nucleoulus appears slightly well rounded with irregular to regular high density granules while the space between the nucleus and nuclear membrane is filled or sprinkled with medium and dense granular material (fig. 1, 4, 5, 9). Morphologic structures in these micrographs also show that highly organised continuous relationship exist between the external nuclear membrane, the cell cytoplasm and the endoplasmic reticulum.

Figure 1a, is a partial longitudinal surface section of the parasite indicating that a circular profile of mitochondrial extension exist between the golgi and the nuclear area. These circular profiles are due to the section and they depict the continuous sinuous elongation of the mitochondria. The mitochondria display its extensive continuity uniting with the agranular secretory reticulum. Distinctive free ribosmes and endoplasmic reticulum loaded with ribosome granules, electron densely stained granular bodies, vacuoles and spherical inclusions are numerously scattered throughout the cell with greater concentration between the nuclear material and the polarized golgi zone (fig. 1a, 1b, 3, 4, 5, 8). The dense bodies are sigle membrane bounded. Similar bodies have been found in *Trypanosoma rhodesiense* and *Trypanosoma equiperdum* (Riley, 1964, Bayne et al. 1969).

Examination of figure 1-a also revealed the presence of a parasite with its nucleus protruding into the sinusoid at the top right corner. The liver cell nuclear area contained scattered lipid inclusions, lysosomes, microbodies and hepatocytic figure contained scattered lipid inclusions, lysosomes, microbodies and hepatocytic balloon cells. Trypanosome found inside hepatic sinusoid (fig. 1b) possesses no flagellar bundle attached to the cell, but broken remnants of flagellum and portion of another cell trapped in the sinusoid. A prominent feature of these sinusoid cells are dense bodies, free ribosomes, lysosomes, and abundant and swollen mitochondria (fig. 2, 6). Figure 2 shows joined but inseparable parasite cells and depicts the cells to contain moderately dense nuclear granules, heavy clusters of ribosomes and single membrane bound vessicles, whereas the cell phagocytized by liver Kupffer cell (fig. 6) depicts an inflammation of the cytoplasmic content at the posterior end with membraneous swellings protruding or extending over adjacent parasites.

In the parasites trapped in the liver sinusoid and the space of Disse (fig. 3, 5), only two of the trilaminar membranes are vissible. Traces of gorgeously displayed endoplasmic retuculum which are established in two areas, lower left and right (fig. 3), and upper left and lower right (fig. 5) demonstrate the invagination type source of origin from the cell membrane. The outer nuclear membrane often ramifies into the ribosome-rich cytoplasm of the cell forming cisternae (fig. 3, 5). Liver parenchyma cells and the parasite contain many lysosomes, variable size dense bodies and single membrane vacuoles. In addition, unusually excessive deposits of ferritin clusters are found in the liver cells, in the sinusoid and on numerous microvilli covering the surface of Disse space. Many deposits are also found around the endothelium cell fenestrations to which the parasite is attached, and blocking them in its position of contact (fig. 3).

Survey of magnified micrographs of the posterior region (fig. 7, 9) demonstrates the polarized region of the golgi, the region of flagellar pocket and the portion of the anterior area past the nucleus. Cytoplasmic structural elements in the cell seem to show close affinity with the flagellar pocket, membraneous evagination of the region, free ribosomes in kinetoplast,s vicinity, lysosomes and external mitochondrial membrane clearly in vissible contact with the sub pellicular microtubules.

Discussion

Ultrastructure of *Trypanosoma lewisi* in liver tissue share some of the features found in the studies of free suspension in arrangement of sub pellicular microtubules and mastigont system. Single row of microtubules are numerously found in the posterior and middle region of the cell. They are less demonstrable in many of the anterior regional sections examined. Nontheless, variations in number and length of subpellicular microtubules have been reported (Angelopoulos, 1970).

The function of the three microtubules which form a cluster beneath the limitting membrane of the flagellar pocket is not clear. Detection of similar microtubules have been reported (Anderson and Ellis 1965, Burton 1966, Rudzinska and Vickeman 1968, Taylor and Gogfrey 1969). However Angelopoulos (1970) suggested that they may

have a role in attaching the flagellum to the pellicular microtubules. Vickerman (1969) also found such microtubules beneath the points of attachment of the flagellum *maculae adherens* in *Trypanosoma congolense.*

Deep in the cell,s cytoplasm, occurrence of four largely restricted vacuole-like tubules have been demonstrated, that is on the right side of kinetosomal plate and kinetosome. Two of them contain few stained granular inclusions. Their functional role though may not be related to impulse coordination and cytoskeleton maintenance, it may not preclude their involvement in pinocytotic activity. It is not certain whether their formation may not indicate an active undulation of the membrane.However, in *Trynaposoma lewisi* few subpellicular tubules connect with the flagellar tubules in the region of flagellar complex (Anderson and Ellis 1965). The stained inclusions may be concentrated vacuolar materials from vacuolization process. They could also be participating in the endocytotic process associated with the digestive apparatus of the cell involving lysosomes. Paulin (1969) found such cytoplasmic microtubles in *Cristhiadia fasciculata* and in *Phytomonas* during early stages of division. He postulated that since their appearances are on the lateral sides of kinetosomes and not between the two kinetosomes, they may have some functions in spacing of the daughter kinetosomes and prokinetosome.

The cross striated pattern of intra-flagellar system which has a honey-comb appearance undoubtedly participate in rigidity maintenance of the entire flagellar structure. Observations of flagellar in this study (fig. 5, 7) demonstrate the existence of complete separation of kinetosome from the flattened kinetosomal plate which by position preceed the kinetosome proper. Adjacent to these two is a new prokinetosome. The localization of prokinetosome and the separated kinetosomal plate in these positions seem significantly related to elongation process of the entire flagellar complex and may indicate that kinetosome replication is initiated beyond kinetoplast division (fig. 5, 7). This finding is supported by observation of stages of division in *Phytomonas elmassiani* which showed kinetosome replication preceeds kinetoplast division, where elongation was detected to be in juxtaposition to the kinetosome (Paulin and McGhee 1971). The occurrence of a pair of basal plate at different elevation at this particular juncture reported by Anderson and Ellis (1965) could not be located in this study.

Accessory filaments of *Trypanosoma brucei* sub-group possess a lattice-like ultrastructure. The doublet pattern of pronounced peripheral tubules which was found in the flagellar organelle was also demonstrated by Vickerman (1962). In this report, figures 8,2 and 7 suggest that the intra flagellar structure is composed of flattened sheets which run in a honeycomb pattern transversely or obliquely into dense amorphous matrix. The absence of this structure in the flagellar of some trypanosomes confirms that it is not essential for flagellar contraction. But Trager (1963) and Anderson and Ellis (1965) have demonstrated the occurrence of this intra-flagellar rod-like structure in two issolates of *Trypanosoma lewisi* and *Leishmania donovani.*

The mitochondrial extension from the kinetoplast deep into the cytoplasm (fig. 5, 6) confirms the report of Anderson and Ellis (1965) that it originates from the kine-

toplast. Ultrastructure of the parasite in liver cells (fig. 5, 7) study showed that the cell's cytoplasm contains rough surfaced endoplasmic reticulum stretching far posteriorly to the golgi complex and all the way to the posterior end of the kinetoplast, with free ribosomes and dense basophilic granules which convincingly indicate that the organism is progressively synthesizing protein.

As far as it is known, no literature report on the ultrastructure of *Trypanosoma lewisi* in liver tissue. The present report shows that *Trypanosoma lewisi* is found in liver hepatic sinusoids and space of Disse in infected rat. Sanabria (1970) found *Trypanosoma cruzi* only in hepatic sinusoids and inside Kupffer cells. Reports of electron microscopic studies indicate that malaria and kala azar parasites have been found inside Kupffer cells and not in the hepatocytes (Miwa and Tanikawa 1965, Rosen et al. 1967, Tanikawa and Hojiro 1965). Electron microscopic observations in this studies show that when trypanosomes where found enclosed in hepatic sinusoid and space of Disse, some alterations of the parasite occurred. Balloon size inflammations or swellings of the flagellum at the base, the trilaminar cell membrane and the cytoplasmic content of the posterior end of the organism are also displayed.

The electron micrographs also showed the posterior portion of the cell with a stumpy remnant of the undulatin flagellar still attached. Broken pieces of flagellum in the vissinity of trypanosome cells have been demonstrated inside the hepatic sinusoid (fig. 1a, 7). Main causes of alterations or swellings are not directly known, but they might have their origin in the cell's increased rhythmic activity of locomotion within liver tissues coupled with the effect of hydrolytic enzymes of numerous lysosomes of liver cells.

Electron micrographs of this work have also revealled the occurrence of increased fine clusters and dense granular materials, vacuoles of different sizes, dilated cisternae of rough endoplasmic reticulum, swollen mitochondria and variable droplets of fatty infiltrations in the liver cells and to an extent in the attached parasites. Previous workers have reported the presence of pathological alterations in the liver tissues of the host infected with leshmania bodies, *Trypanosoma brucei* and *Trypanosoma cruzi* (Tanikawa and Hojiro 1965, Seneca 1969, Wrights et al. 1969, 1970, Sanabria 1970). In this investigation, the alterations seen in liver cells cannot be due alone to the presence of the parasite restricted to their interior. However, it is possible that excessive accumulation of metabolic products from the parasite may have exerted toxic alterable effects on the liver cells where the parasites are located.

Key for Abreviations Used in the Figures

AR, anterior region ; AT, attachment zone of flagellar ; CM, cell membrane ; CMJ, cell membrane juncture ; CR, cristae ; DB, dense body ; EC, endothelial cells of sinusoid ; ER, endoplasmic reticulum ; F, flagellar ; FI, flagellar infrastructure ; FR, free ribosome ; FP, flagellar pocket ; G, golgi apparatus ; HC, hepatic cell ; K, kinetoplast ; KM, kinetosome ; KP, kinetosomal plates ; L, lipid ; LM, liver microvilli ; LY, lysosomes or microbodies ; M, mitochondria ; Mt, microtubules ; MV, membrane bound vessicle ; N, nucleus ; NU, nucleolus ; NP, nuclear pore ; PK, prokinetosome ; PL, blood platelets ; PR, posterior region of parasite ; RBC, reb blood cells ; RV, agranular secretory reticulum ; SD, space of Disse in the liver ; SN, sinusoid of liver ; VB, vessicular bodies.

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ERRATUM

Effets of Pregnancy on *Trypanosoma congolense*. Infection in Rats : Serum Biochemistry and Cellular Disorders, by J. O. SIMAREN and J. I. AWOPETU.

In this work published in our issue n° 3, May/June 1973, page 436, first paragraph, line 6-7, delet the sentence : « Trypanosoma and blood cell counts were performed show and water ad libitum. »

At the same page, the second sentence of paragraph 2, should be read as follows : « These tests were performed on the 10th day after infection. »